

Appl. No. 10/656,185  
Amendment dated August 19, 2004  
Reply to Office Action of March 19, 2004

### **IN THE SPECIFICATION**

Please replace the title of the invention with the following amended title:

## **SUPERSENSITIVE NUCLEAR MAGNETIC RESONANCE MICRO IMAGING APPARATUS**

**Please replace the paragraph on page 16, lines 9-20, with the following amended paragraph:**

The first embodiment of the present invention is shown in Fig. 1. In the superconducting magnets 1, 2, 3, the coils are formed in such a manner that the inner side, that is the side closer to the sample, is formed of a material having a higher superconducting critical magnetic field. For example, the superconducting magnet 1 is formed of Nb<sub>3</sub>Al, the superconducting magnet 2 is formed of Nb<sub>3</sub>Sn, and the superconducting magnet 3 is formed of NbTi; and, they can be optimally combined to obtain desired values of coil-generated magnetic field and of uniformity as needed. For example, a Bi-type such as Bi<sub>2</sub>Sr<sub>2</sub>CaCu<sub>2</sub>O<sub>9</sub> and the like, or superconducting material, such as Y<sub>1</sub>Ba<sub>2</sub>Cu<sub>3</sub>O<sub>7</sub> and the like, may be used, or MgB<sub>2</sub> and the like may also be used. The direction of generation of the magnetic fields of the superconducting magnet 1, 2, 3 constructed of a combination thereof is horizontal.

**Please replace the paragraph spanning pages 16 and 17 with the following amended paragraph:**

In Fig. 1, the biosample 4, such as small animals, cells, and organic tissues, is inserted from the top of the apparatus into the center of the magnetic field in a

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vertically oriented glass container having magnetic properties equivalent to water and which is 1 to 30 mm in diameter. The magnetic field is applied to the sample 4 in the lateral direction from the side. Therefore, each superconducting magnet 1, 2, 3 is wound in the state of a solenoid with the axis thereof oriented in the horizontal direction, and which is disposed laterally symmetrically. The maximum width of the magnet 1, 2, 3 is 400 mm, and the maximum height is 700mm, which will allow for a compact arrangement.

**Please replace the paragraph on page 17, lines 4-10, with the following amended paragraph:**

At the center of the magnet 1, 2, 3, the uniformity of the magnetic field is adjusted to a value not greater than 0.001 ppm, which corresponds to 0.5 Hz or less when represented by Proton nuclear magnetic resonance frequencies, and the time based stability is not greater than 0.001 ppm/h, which corresponds to 0.5 Hz/h or less, when represented by Proton nuclear magnetic field resonance frequencies. In this case, a coil for uniformity adjustment may be disposed in the vicinity of the center of the magnetic field as needed.

**Please replace the paragraph on page 17, lines 11-20, with the following amended paragraph:**

A conducting wire may be used for adjustment at a constant temperature portion, or another superconducting wire may be used for adjustment at the lower temperature portion, or a combination thereof may be used for adjustment. For example, when it is used for NMR of 600 MHz in the Proton nuclear magnetic

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resonance frequencies, the central magnetic field is generally 14.1 T and the uniformity of the magnetic field is not greater than 0.5 Hz in the sphere of 18 mm when represented by the Proton nuclear magnetic resonance frequencies. Under such conditions, the operating temperature of the coil is 4.2 K and the pumping of liquid helium is not necessary, whereby the operation is easy. The sample 4 is inserted vertically.

**Please replace the paragraph spanning pages 17 and 18 with the following amended paragraph:**

According to this method, since the magnetic fields are generated in the horizontal direction and the sample 4 is inserted from the top in the vertical direction, there is no possibility that a solution, such as organic tissues in a test tube, will spill over. Further, since it is constructed so that the detector coil 5 can be inserted from below, a sufficient space for a sample 4 can be secured, and thus the sample space can be used as efficiently as possible for a measurement that requires a given sensitivity for analyzing a biosample 4. Even when the detector coil system is cooled to low temperatures, such an arrangement allows various conditions in imaging of the information network of a protein in living organisms to be easily changed.

**Please replace the paragraph on page 18, lines 12-22, with the following amended paragraph:**

The superconducting magnet 1, 2, 3 is held at the permanent current mode by the permanent current changeover switch 9, and time-based variations of the magnetic field are adjusted to 0.5 Hz per hour or less. The superconducting magnet

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1, 2, 3 is soaked in liquid helium 7 and held at low temperatures. Consumption of helium is cut down by employing a double structure in which the outside is covered by liquid nitrogen 8. It is also possible to employ a refrigerating machine that has no vibration problem, such as a pulse tube refrigerator, to cool the superconducting magnet directly, instead of cooling it down by use of liquid helium 7. Reduction of leakage of the magnetic field around the magnet 1, 2, 3 is important from the viewpoint of the installability and safety of the apparatus, and so a magnet structure including a magnet shield is realized that meets the installation conditions.

**Please replace the paragraph on page 19, lines 7-17, with the following amended paragraph:**

In the supersensitive nuclear magnetic resonance imaging method according to the present invention, when the nuclear magnetic resonance frequency is 14.1 T (600 MHz), for example, the chemical shift of a Proton, or the line width, is approximately 10 ppm, which corresponds to 6000 Hz, and thus resolutions on a  $\mu\text{m}$  scale could not be obtained without modification. Therefore, the resolution was increased by spectrally factorizing the Proton NMR before imaging. In other words, a gradient magnetic field was applied to give positional information to a nuclear spin, and then the Proton NMR spectrum was measured to obtain a two-dimensional image of a specific spectrum line. Since the line width of the specific spectrum line was in the order of ~~0.01 ppm~~ 0.1 ppm (0.014 G), the spatial resolution could be drastically increased.

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**Please replace the paragraph spanning pages 19 and 20 with the following amended paragraph:**

When a gradient magnetic field of 280 G was given to the sample space of 10 mm, and the spatial distribution of a specific spectrum line was observed, a spatial resolution of 0.5 micron could be obtained. In order to enable such measurement, the magnetic field uniformity of 0.001 ppm was required in the measurement space of 600 MHz (14.1T), and, in addition, an effective accumulation of a faint spectrum by the supersensitive measuring method was required; and, thus the solenoid signal detector coil 5 of the present invention was essential. This enabled observation of a metabolic reaction of protein in the cell by imaging. The five gauss line of the magnetic field leaked from the apparatus was 2 m in the vertical direction and 3 m in the horizontal direction at the maximum. This enabled installation of the apparatus without providing a specific building.

**Please replace the paragraph on page 20, lines 4-13, with the following amended paragraph:**

The second embodiment of the present invention is shown in Fig. 2. In this embodiment, the construction is generally identical to the first embodiment, but the low temperature container is divided by the left and right superconducting magnets to provide an openness to the available space for the user. In other words, since there is an open space 10 around the sample chamber, unlike the hermetical sample space in the previously known construction, the dynamic behavior of the living organisms, such as photosynthesis, can be measured, while performing light irradiation or laser beam irradiation on the sample 4 easily. Since the dynamic NMR

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signal can be observed in this manner, for example, the signal transmission or reaction of photosynthesis of the protein can be inspected.

**Please replace the paragraph spanning pages 20 and 21 with the following amended paragraph:**

When performing such special experiments, liquid helium 7 is pumped to cool down and operate the apparatus at 1.8 K, so that the superconducting magnet 1, 2, 3 can be operated approximately at 900 MHz (21.1 T) at the central magnetic field. The detection sensitivity in this case is equivalent to the NMR of 2 GHz or higher when converted to the previously known NMR apparatus shown in Fig. 8, which significantly exceeds the critical magnetic field of the traditional superconducting material. Therefore, though such detection sensitivity could not be achieved heretofore, a high level of detection sensitivity, which could not previously be achieved, can be realized easily at magnetic field strengths between 600 and 900 MHz (21.1T). In this case, as well, the magnetic fields that leaked from the apparatus in the vertical direction are significantly different from those in the case of the NMR apparatus of 900 MHz class in the previously known apparatus, and, in accordance with the present invention, it was 3 m in the vertical direction, and 4.5 m at the maximum in the axial direction of the coil (horizontal direction).

**Please replace the paragraph on page 21, lines 4-11, with the following amended paragraph:**

A third embodiment of the present invention is shown in Fig. 3. In this embodiment, the biosample 4 is inserted into the apparatus from the top, and the

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measurement probe is inserted from the side. In this arrangement, the frame 11, that is provided with an anti-vibration device, can be lowered; and, thus the height of the apparatus can be lowered, whereby the operability is improved, and vibrations propagated from the floor to the apparatus can be reduced. Therefore, an economically effective system, which is superior in installability and maintainability, can be provided.

**Please replace the paragraph on page 21, lines 13-20, with the following amended paragraph:**

The fourth embodiment of the present invention is shown in Fig. 4. In this embodiment, the biosample 4 is inserted into the apparatus from the side, and the measurement probe is inserted through the through hole 12 formed on the other side. In this arrangement, the height of the apparatus can be lowered so that it can be used even when there is a limit in the height of the ceiling, whereby the operability is significantly improved and vibrations propagated from the floor to the apparatus can be reduced. Therefore, an economically effective system, which is superior in installability and maintainability, can be provided.

**Please replace the paragraph spanning pages 21 and 22 with the following amended paragraph:**

The fifth embodiment of the present invention is shown in Fig. 5. The biosample 4 ~~[[is]]being sent to the sample being-chamber, is~~ maintained in a living state by the use of an oxygen supply unit 13. The NMR signal is sent to the controller 15 via a preamplifier 14. Various types of pulse sequences are applied to

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receive the NMR signal. The pulse is emitted as needed, and the pulse sequence is combined with a gradient magnetic field and imaged, and then sent to the display 16. It is also possible to use the preamplifier 14, after being cooled to temperatures close to that of liquid nitrogen, in order to reduce heat noise in the signal.